SEPARATION OF TWO INITIATOR TRANSFER RNAs FROM *E.COLI*

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Recently the sequence of the bacterial initiator tRNA, $tRNA_f^{Met}$, was elucidated [1]. During the course of the sequence work a minor $tRNA_f^{Met}$ was detected having an A residue instead of a 7MeG residue at position 31 numbered from the amino acid acceptor end. The relative proportions of the two $tRNA_f$ species were estimated as approximately 75% with a 7MeG and 25% with an A. Evidence was further presented to show that this base change was probably the only difference between the two tRNAs. This communication describes the partial separation of these two $tRNA_f$ species and demonstrates that the two species have identical coding properties.

$tRNA_f$ labelled with ^{32}P was first separated by chromatography on DEAE Sephadex as described elsewhere [2]. Subsequently the purified $tRNA_f^{Met}$ species were subjected to chromatography on benzoylated DEAE cellulose [3]. Fig. 1 shows the result of such an experiment. The first eluted peak (A) containing the $tRNA_f$ activity is asymmetrical, suggesting a partial separation of two isoaccepting species, $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$. For this reason $tRNA_f^{Met}$ was isolated from fractions at the indicated positions of the elution profile. After their isolation samples from the two fractions were digested with T1 or pancreatic ribonuclease. The resulting enzymic digests were then fractionated by the usual two-dimensional system [4] using 7% formic acid in the DEAE paper dimension. Fig. 2 shows a comparison of the T1 ribonuclease "fingerprints" of the respective $tRNA_f$ fractions. Fig. 3 shows a similar comparison of the pancreatic ribonuclease "fingerprint". It is evident that $tRNA_{f1}^{Met}$ from fraction 1 contains relatively small amounts of the 7MeG-containing sequence (about 30%), while having a high proportion of the corresponding A-containing sequence (about 70%). The $tRNA_{f2}^{Met}$ from fraction 2, on the other hand, contains very little of the A-containing sequence (< 10%), but gives almost molar yield of the 7MeG-containing sequence. Thus, the two $tRNA_f^{Met}$ species have been partially separated from one another, the 7MeG-containing $tRNA_{f2}^{Met}$ being almost completely separated from the other $tRNA_f^{Met}$. Further comparison of the respective T1 and pancreatic ribonuclease "fingerprints" of the two $tRNA_f$ fractions revealed that all other nucleotides were identical and present in equal yields. Thus the two $tRNA_f^{Met}$ species differ only by one base change at the indicated position.

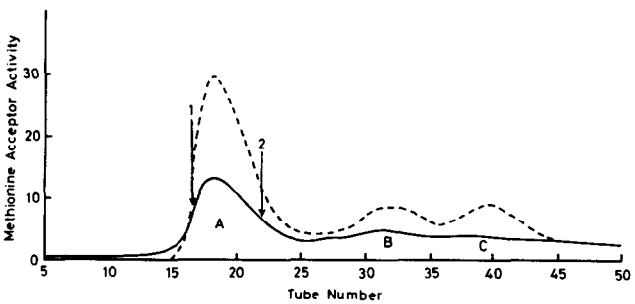

Fig. 1. Chromatograph of purified $tRNA^{Met}$ species on benzoylated DEAE cellulose. The column was developed by a linear gradient formed from 100 ml of a solution containing 0.45M NaCl, 0.01M $MgCl_2$, 0.001M 2-mercaptoethanol and 100 ml of a similar solution containing 0.9M NaCl. 1.5 ml fractions were collected. Methionine acceptance was determined as described elsewhere [2]. Peak A contains $tRNA_f^{Met}$, while peak C contains $tRNA_m^{Met}$. Peak B was not rigorously identified but "fingerprints" of T1 and pancreatic ribonuclease digests of material from this peak showed that it contained $tRNA_f^{Met}$. The solid curve represents ^{32}P cpm, whereas the dotted curve refers to methionine acceptance in arbitrary units.

The codons for $tRNA_f^{Met}$ have been shown to be AUG and GUG, therefore the coding properties of

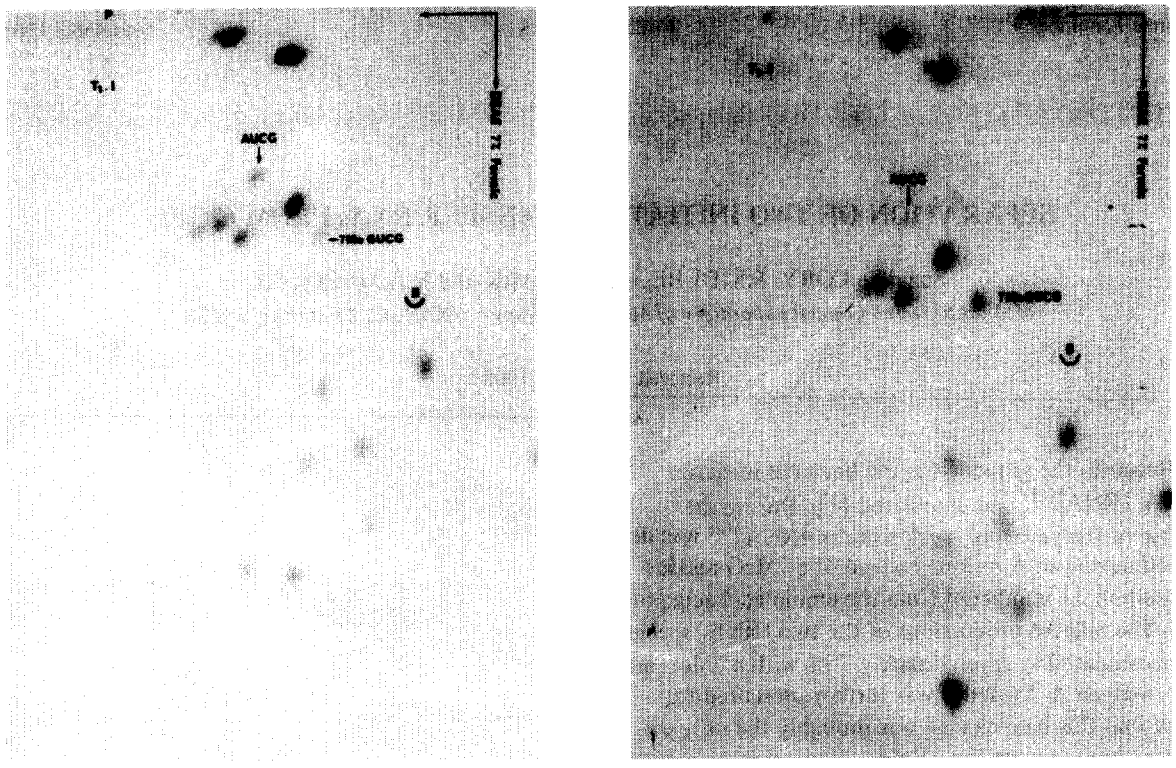

Fig. 2. A two-dimensional fractionation of ribonuclease T1 digests of fraction 1 and 2 respectively. The presence of double spots, especially in the "fingerprint" of fraction 1, $tRNA_{f1}^{Met}$, due to the cyclic isomers, is because of slightly incomplete digestion. P and B show the positions of pink and blue markers respectively.

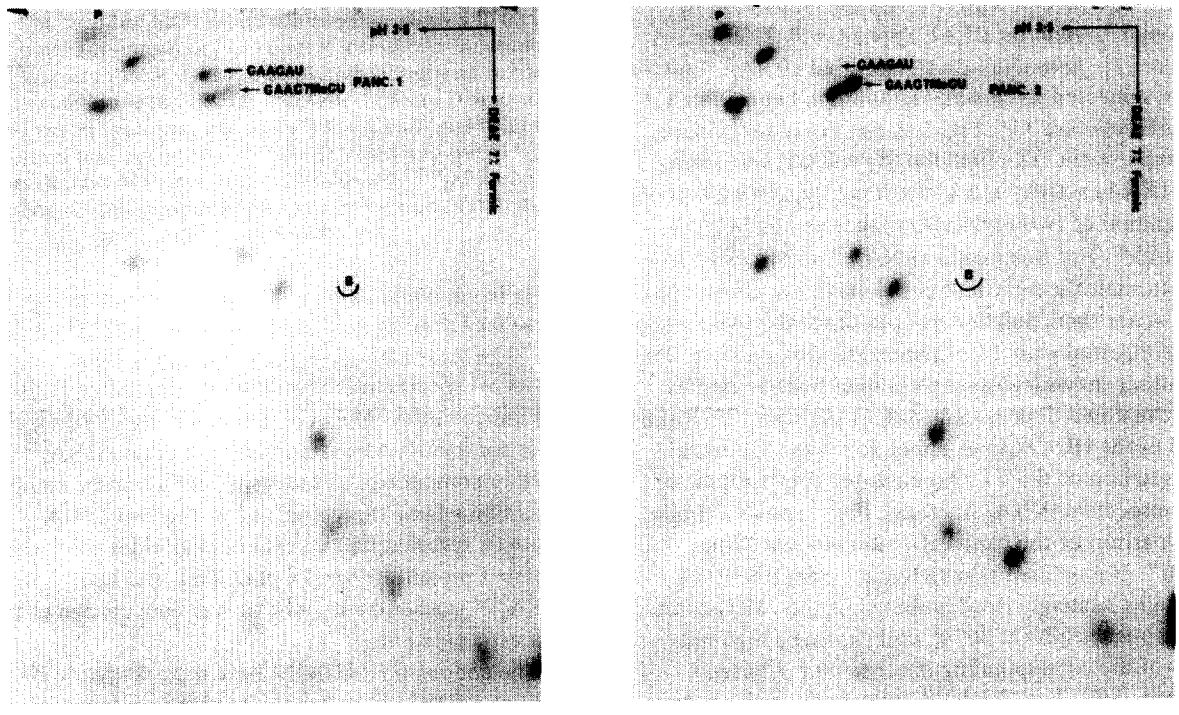

Fig. 3. A two-dimensional fractionation of pancreatic ribonuclease digests of fraction 1 and 2 respectively. P and B refer to the positions of pink and blue markers.

Table 1
Coding of ^{32}P-tRNA$_f^{Met}$ species.

Triplet added	tRNA$_{f1}^{Met}$	tRNA$_{f2}^{Met}$
(A_{260} units)	cpm bound	
None	115	81
AUG (0.21)	640	390
GUG (0.20)	390	200

The reaction mixture (50μl) contained 0.01M Mg acetate, 0.05M K chloride, 0.1M Tris HCl pH 7.2, 0.8 A_{260} units of *E. coli* MRE600 70S ribosomes, 1740 cpm of ^{32}P-tRNA$_f^{Met}$ species and triplet as indicated. Incubation was for 20 min at 22°C.

the two species were investigated using the Nirenberg-Leder binding assay technique [5]. Table 1 shows the result of a typical experiment. No significant difference in codon response was detected, each tRNA$_f^{Met}$ species responding to both AUG and GUG. It has already been shown that the anticodon loop of tRNA$_f^{Met}$ also responds to both the codons AUG and GUG [6]. Thus the dual codon recognition of tRNA$_f^{Met}$ is not due to additive coding of separate tRNA$_f^{Met}$ species but rather that it represents a unique feature of tRNA$_f^{Met}$.

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